In vivo mechanisms of uterine myoma volume reduction with ulipristal acetate treatment

Guillaume E. Courtoy, M.Sc.,[a] Jacques Donnez, M.D., Ph.D.,[b] Etienne Marbaix, M.D., Ph.D.,[c,d] and Marie-Madeleine Dolmans, M.D., Ph.D.[a,e]

[a] Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain; [b] Société de Recherche pour l'Infertilité; [c] Cell Biology Unit, de Duve Institute, Université Catholique de Louvain; [d] Pathology Department, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain; and [e] Gynecology Department, Cliniques Universitaires St-Luc, Brussels, Belgium

Objective: To study the in vivo mechanisms of action of ulipristal acetate (UPA) on uterine myomas.
Design: Retrospective histologic and immunohistochemical (IHC) study of myomas.
Setting: Academic research unit.
Patient(s): Among 59 women with symptomatic myomas who underwent myomectomy, 42 were treated preoperatively with UPA, while 17 were not.
Intervention(s): Histology and IHC were analyzed on tissue microarrays obtained from surgical specimens.
Main Outcome Measure(s): Proliferation, apoptosis, extracellular matrix (ECM) remodeling, and matrix metalloproteinase 2 (MMP-2) expression.
Result(s): Proliferation was low in all conditions, with no statistical difference between groups. Terminal deoxynucleotide transferase–mediated dUTP nick-end labeling assay showed an increase in cell death in UPA-treated myomas compared with untreated myomas, but only after short-term treatment; this was not associated with elevated levels of cleaved caspase-3. After long-term treatment, cell density was higher and the ECM volume fraction lower in UPA-treated myomas than in untreated myomas. MMP-2 expression was found to be increased after treatment, showing the highest level after long-term treatment, compared with untreated myomas.
Conclusion(s): Regarding sustained clinical volume reduction of myomas, this study strongly points to multifactorial mechanisms of action of UPA, involving: 1) a persistently low cell proliferation rate; 2) a limited period of cell death; and 3) ECM remodeling concomitant with stimulation of MMP-2 expression. (Fertil Steril® 2015;104:426–34. ©2015 by American Society for Reproductive Medicine.)
Key Words: Uterine myoma, ulipristal acetate, proliferation, cell death, extracellular matrix, matrix metalloproteinase 2

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Uterine myomas are the most commonly observed benign neoplasms, with a high prevalence among premenopausal women (as high as 70% at 45 years of age) (1, 2). Symptoms manifest in 15%–30% of cases (3). This incapacitating pathology is responsible for complications, such as menometrorrhagia, leading to anemia, pelvic pain, and miscarriage, depending on the number, volume, and localization of myomas in the uterus (4). They constitute the first indication for hysterectomy (5). For patients wishing to conceive, conservative surgery may be offered, and recent technical improvements (laparoscopic laser-assisted myomectomy) have contributed to reducing morbidity rates (6).

The etiopathology of transformation from myometrial to myoma cells is poorly understood, but tentatively associated with complex networks of multiple factors: genetic and epigenetic alterations, hyperresponsiveness to growth factors and steroid hormones, cell cycle perturbations, and extracellular matrix (ECM) dysregulation (7, 8). Myomas are considered to be monoclonal tumors arising from single somatic stem cells of the

Received January 26, 2015; revised March 25, 2015; accepted April 20, 2015; published online May 21, 2015.
G.E.C. received a grant from Preglem (Geneva, Switzerland) for this original work. J.D. has been a member of the Scientific Advisory Board of Preglem since 2007. E.M. has nothing to disclose. M.-M.D. has nothing to disclose.
Reprint requests: Marie-Madeleine Dolmans, M.D., Ph.D., Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Avenue Mounier 52, bte B1.52.02, 1200 Woluwe-Saint-Lambert, Brussels, Belgium (E-mail: mari-madeleine.dolmans@uclouvain.be).

Fertility and Sterility® Vol. 104, No. 2, August 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.04.025

myometrium, as suggested by earlier genetic studies (9, 10), bearing a resemblance to catastrophic rearrangement of chromosomes that may be at the origin of the disease, although myoma cells are TP53 positive (10). This transformation often occurs in multiple sites (8), each myoma possibly arising from different mutations, and patients treated with myomectomy are prone to develop other myomas (11).

Myoma growth requires high levels of both estrogen and progesterone, and mainly occurs when progesterone peaks, usually regressing with menopause (12, 13). Although estrogen stimulates expression of progesterone receptors (PRs) (14), progesterone is the major promoter of myoma growth (14, 15).

Ulipristal acetate (UPA), a selective PR modulator (SPRM), is characterized by its superior selectivity for PRs, even higher than progesterone itself (16, 17). Depending on the tissue, SPRMs exert an agonistic or antagonistic effect; binding to PRs, they allow recruitment of corepressors or coactivators according to the cellular context (18–20). Clinically, UPA acts swiftly to stop bleeding and reduce myoma size, with a remarkably sustained effect even after treatment cessation (21, 22). A recent long-term study has demonstrated this latter property, the effect being maximized with the use of repeated courses (23).

Some in vitro studies on myoma cell cultures have suggested that multifactorial combinations of decreased proliferation (24), stimulated apoptosis (24), collagen synthesis repression (25), and stimulation of ECM resorption (25) may be implicated, but in vivo, the mechanism of action of UPA on myomas is not yet understood. The aim of the present study was to elucidate the in vivo mechanisms involved in myoma volume reduction after UPA treatment.

MATERIALS AND METHODS

Patients and Tissues

Tissue samples analyzed in the present study were collected from patients given UPA in the course of two clinical studies: PGL4001 Versus GnRH-Agonist in Uterine Myomas (PEARL-II) (22) and PGL4001 Efficacy Assessment in Reduction of Symptoms Due to Uterine Leiomyomata (PEARL-III) (23). The study population included women aged 18–50 years with symptomatic myomas. They were eligible for the study if their myomatous uterine size was equivalent to that of a uterus at $\leq$16 weeks of gestation, and with the presence of at least one fibroid $\geq$3 cm but $<$10 cm in diameter, as evaluated by ultrasonography or magnetic resonance imaging. The patients were randomized and received 5 mg/d or 10 mg/d UPA (see below). In the present study, mean age ($\pm$SD) was 37 ($\pm$11) years, and distribution of race was black (37%), white (45%), North African (8%), and other (10%). We distinguished three regimens of UPA treatment, constituting three study groups: 1) 5 mg/d UPA for 12 weeks (UPA5; n = 10) (22); 2) 10 mg/d UPA for 12 weeks (UPA10; n = 13) (22) (both considered to be short-term treatment (UPA-ST)); and 3) 10 mg/d UPA for at least 2 and up to 4 cycles of 12 weeks, each separated by an off-treatment period including a full menstrual cycle up to the start of the second menstruation (UPA10-LT; n = 19; considered to be long-term treatment) (23). Patients undergoing UPA treatment did not take any other medication. The control group comprised 17 women with symptomatic myomas who had not received any therapy (untreated; UPA0). All patients underwent surgery, and the resected myomas were fixed in 4% formalin and embedded in paraffin. Only intramural fibroids from 3 to 10 cm in diameter were included in the present study. Use of human tissue from the biolibrary was approved by the Institutional Review Board of the Université Catholique de Louvain (IRB, 2008, 150; EudraCT number 2008/001805-40).

Tissue Microarray

Tissue microarrays (TMAs) were performed as described for myomas to allow multiple comparisons of large numbers of tissue specimens (26). In total, 299 tissue samples (fibroids and myometrium) taken from 59 patients were analyzed for this study. Indeed, to take into account tissue heterogeneity, from 1 to 8 representative 600-μm-diameter samples were taken from paraffin-embedded tissue with the use of a dermal punch biopsy needle and placed in a recipient tissue microarray (TMA) paraffin block. Then, from each assembled TMA block, $>$250 serial sections were cut at 5-μm intervals.

Histology and Immunohistochemistry

Every tenth slide of the TMA sections was stained with hematoxylin-eosin (Merck) for histology; the others were used for specific staining or immunohistochemistry (IHC). Dewaxing and rehydration were performed with the use of serial baths of Histosafe (Yvsolab), alcohol, and demineralized water. Masson trichrome and Picro-Sirius Red staining with Fast Green counterstaining were performed as previously reported (27, 28).

IHC was done as described (29). Briefly, after dewaxing, rehydration, and membrane permeabilization, endogenous peroxidase activity was blocked with the use of 3% (v/v) H_2O_2. Epitope unmasking was performed at 98°C for 75 minutes with the use of citrate buffer (pH = 5.6) in a water bath. Slides were first incubated with blocking solution (decomplemented nonimmune goat serum 10% + bovine serum albumin 1%) and, after extensive washing, with primary antibody (in blocking solution at 4°C overnight). Antirabbit/mouse secondary antibodies conjugated to horseradish peroxidase (Envision+; Dako) were incubated (1:2 dilution in blocking solution) at room temperature for 1 hour. Peroxidase activity was revealed with the use of 3,3′-diaminobenzidine (Dako), and the slides were counterstained with hematoxylin (Merck). The following markers were selected to assess caspase-dependent apoptosis, cell proliferation, and ECM remodeling, respectively: cleaved caspase-3 (G748 A, 1:200; Promega), Ki-67 (M7240, clone MIB1, 1:100; Dako), and matrix metalloproteinase 2 (MMP-2; MAB3308, clone 42–5-D11, 1:500; EMD Millipore [30]). Negative control samples were obtained by omission of the primary antibody or by incubation with an irrelevant same-isotype antibody at the same concentration. Human tonsil or proliferative/menstrual endometrial tissue served